



PATENT
Attorney Docket No. **KNAUTHE-08178**

LISTING OF THE CLAIMS

- 1-31. Canceled.
32. (New) A cell culture exhibiting cell-type specific or development-specific expression of a non-cell damaging fluorescent protein comprising embryoid bodies formed by aggregates of embryonic stem cells stably transfected with a DNA construct comprising:
 - a) a DNA sequence coding for said non-cell damaging fluorescent protein; and
 - b) a promoter operably linked to said DNA sequence, said promoter selected from the group consisting of a cell-type dependent promoter, a development-dependent promoter and combinations thereof, wherein said promoter is substantially inactive in undifferentiated embryonic stem cells.
33. (New) The cell culture of claim 32, wherein said stem cells are mouse stem cells.
34. (New) The cell culture of claim 32, wherein said aggregates are obtained by the hanging drop method.
35. (New) The cell culture of Claim 32, wherein said non-cell damaging fluorescent protein is selected from the group consisting of Green Fluorescent Protein, Red Fluorescent Protein, and Blue Fluorescent Protein.
36. (New) The cell culture of claim 32, wherein said promoter is a promoter specific for heart cells, neurons, glia cells, hematopoietic cells, endothelial cells, smooth muscle cells, skeletal muscle cells, cartilage cells, fibroblasts and epithelial cells.
37. (New) The cell culture of claim 32, wherein said promoter is selected from Nkx-2.5, human alpha-actin, and MLC-2V promoters.

38. (New) The cell culture of claim 32, wherein said promoter is the heart-specific human alpha-actin promoter.

39. (New) The cell culture according to claim 32, wherein said DNA construct comprises further functional elements.

40. (New) The cell culture according to claim 39, wherein said further functional DNA elements are selected from the group consisting of enhancer elements, selectable marker genes, or combinations thereof.

41. (New) The cell culture of according to claim 32, wherein said DNA construct is the plasmid pCX-(a-act)GFP-Neo (DSM 11633).

42. (New) A method for preparing the cell culture according to claim 32, comprising:

a) providing ES cells comprising a DNA construct coding for said non-cell damaging fluorescent protein and a promoter operably linked to said DNA sequence, said promoter selected from the group consisting of a cell-type dependent promoter, a development-dependent promoter and combinations thereof, wherein said promoter is substantially inactive in undifferentiated embryonic stem cells, and starting ES cells from a non-human mammal;

b) introducing said DNA construct into said starting ES cells; and

b) screening for stably transfected ES cells.

43. (New) The method of claim 42, further comprising the step of establishing embryoid bodies from said stably transfected ES cells.

44. (New) The method of claim 43, wherein said embryoid bodies are obtained by the hanging-drop method.

45. (New) The method of claim 42, wherein said introducing is effected by electroporation.

46. (New) The method of claim 42, further comprising culturing said stably transfected ES cells in vitro.

47. (New) A method for the toxicological examination of substances, comprising adding substances suspected of being toxic to cell cultures according to claim 32 and examining the toxicological effects of said substances on said cell cultures using fluorimetric methods.

48. (New) A method for producing a transgenic non-human mammal exhibiting cell-type specific or development-specific expression of a non-cell-damaging fluorescent protein, comprising:

- a) providing ES cells comprising a DNA construct coding for said non-cell damaging fluorescent protein and a promoter operably linked to said DNA sequence, said promoter selected from the group consisting of a cell-type dependent promoter, a development-dependent promoter and combinations thereof, wherein said promoter is substantially inactive in undifferentiated embryonic stem cells;
- b) injecting said ES cells into blastocysts of a non-human mammal;
- c) transferring said blastocysts into a surrogate mother; and
- d) recovering said transgenic non-human mammal from said surrogate mother.

49. (New) The method of claim 48, wherein said transgenic non-human mammal is a mouse.

50. (New) A transgenic mouse obtained by the method of claim 49.

51. (New) A method of using the transgenic non-human mammal of claim 48 for examining stages of development of cells, comprising examining cells of said transgenic non-human mammal in vitro using fluorimetric methods.

52. (New) A method for determining whether a substance influences the differentiation of cells comprising:

- a) providing a substance suspected of affecting development and a cell culture according to claims 32; and
- b) analyzing said cultures to determine which cells are expressing fluorescent protein under conditions such that fluorescence is indicative of differentiation.

53. (New) A method according to claim 52 for determining whether a substance influences the differentiation of heart cells, wherein said analyzing comprises determining the area of heart cells expressing fluorescent protein.